Journal of Medical Bacteriology

Investigation of the Antibacterial Effects, Skin Sensitivity, and Irritant Properties of a Lubricating Gel Containing Silver/Zinc Oxide Nanostructure and Lidocaine in an Animal Model

Alireza Jafari [1], Mohammad Naimi Joubani [2], Marjan Daeihamed [3], Mahan Azizzade Dobakhshari [1], Samira Rasouli Koohi [4]*

1 Urology Research Center, Razi Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

2 Research Center of Health and Environment, School of Health, Guilan University of Medical Sciences.

3 Department of Pharmaceutics, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.

4 Department of Physics, University of Alberta, Canada.

ARTICLE INFO

Article type:
Research Article

Article history:

Received	21	Jun	2025
Revised	05	Jul	2025
Accepted	22	Jul	2025
Published	23	Aug	2025

Keywords:
Irritability, Silver nanostructures, Skin sensitization, Urinary infection, Zinc oxide nanostructures.

*Corresponding Authors: Mahan Azizzade Dobakhshari: Urology Research Center, Razi Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran. *Tel*: +98-13-33525259, *E-mail:* mahanazizzademaz@gmail.com.

ABSTRACT

Background: Urinary tract infections (UTIs), predominantly catheter-associated (CAUTIs), cause significant morbidity (~380,000 cases), mortality (~9,000-13,000 deaths), and healthcare costs annually. CAUTIs initiate via bacterial biofilm formation on catheters. Current antimicrobial catheters (e.g., silver ions, antibiotics) face limitations like poor efficacy, resistance, and non-adjustability. Nanostructure coatings offer promise; notably, combining Ag/ZnO nanostructures leverages enhanced biocompatibility and potent antibacterial effects for novel CAUTI prevention strategies.

Methods: AgNPs were chemically synthesized via silver nitrate reduction (sodium citrate/sodium borohydride); ZnO-NPs were prepared from zinc acetate/sodium hydroxide. A lubricant gel containing 2% lidocaine and Ag/ZnO nanostructures (2:8 v/v ratio) was formulated. Nanostructure characterization included DLS, TEM, and ICP-OES. Antibacterial efficacy was assessed via disk diffusion and microdilution (MIC/MBC) against *S. aureus* and P. aeruginosa. Biocompatibility was evaluated via rabbit ocular irritability and Guinea Pig Maximization Test (ISO 10993-10).

Results: DLS/TEM characterization showed AgNPs (24.4 nm, spherical) and ZnO-NPs (52.48 nm, clustered) were mono-disperse (PDI ~1 and 0.667). Zeta potential confirmed stability (Ag: -20.7 mV; ZnO: -0.709 mV). ICP-OES quantified concentrations (Ag: 25 ppm; ZnO: 970 ppm). Antibacterial assays revealed weak AgNP activity (MIC/MBC >12.5 µg/mL for both pathogens) but potent ZnO-NP effects (e.g., *S. aureus* MIC: 0.406 µg/mL). Ag/ZnO hybrids exhibited enhanced efficacy, especially against *S. aureus* (MIC: 0.02/3.12 µg/mL). The gel was non-irritating (rabbit ocular test) and non-sensitizing (Guinea Pig Maximization Test).

Conclusion: The active formulation consisting of mixed silver and zinc oxide nanostructures combined with a medical lubricant containing 4% lidocaine demonstrates effective antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while exhibiting no irritant or sensitizing properties in laboratory animals.

- ***Please cite this paper as:*** Jafari A, Naimi Joubani M, Daeihamed M, Azizzade Dobakhshari M, Rasouli Koohi S. Investigation of the Antibacterial Effects, Skin Sensitivity, and Irritant Properties of a Lubricating Gel Containing Silver/Zinc Oxide Nanostructure and Lidocaine in an Animal Model. *J Med Bacteriol.* 2025; **13** (3): pp.91-104. DOI: 10.18502/jmb.v13i3.19512

Introduction

Urinary tract infection (UTI) is one of the most common healthcare-associated infections (1, 2). Approximately 70 to 80 percent of these infections are associated with the use of urinary catheters (1). According to reported data, catheter-associated UTIs account for more than 380,000 new cases and 9,000 to 13,000 deaths annually. UTI complications have led to increased patient dissatisfaction and prolonged hospital stays, resulting in higher healthcare costs. Catheter-associated UTIs begin with microbial attachment and colonization on the catheter surface, leading to the formation of a microbial biofilm. In this stage, bacteria ascend towards the bladder, causing infection spread (3).

Various methods have been investigated to address the problem of UTIs and catheter-associated urinary tract infections. One of these methods is the production of antimicrobial coatings or antibiotic coatings containing silver ions, water-repellent substances, cationic ions, and antibacterial and antimicrobial agents on urinary catheters to prevent bacterial attachment. Nowadays, biocompatible polymers such as polycaprolactone, poly (lactic acid), poly (ethylene glycol)-block-poly (D, L-lactide), and poly (ethylene glycol) block-poly(e-caprolactone) are widely used in medical applications as biodegradable materials (4).

In an ideal scenario, catheters remaining in the body should have a stable antimicrobial effect and compatibility, which can be adjusted according to the occurrence and levels of CAUTIs (catheter-associated urinary tract infections). Currently, this mainly relies on the intrinsic antibacterial properties used in antimicrobial catheters to prevent CAUTI. Silver ion hydrophilic coatings and antibiotic washing solutions are common antibacterial methods for clinical catheters. However, hydrophilic silver ion catheters cannot be adjusted according to the conditions of infection and, clinically, their antimicrobial effect does not seem significant. Antibiotic wash solutions have shown improvements, but issues such as antibiotic resistance and rapid release and reduction of antibacterial effects are major concerns (5).

One promising antimicrobial approach involves catheters coated with antimicrobial peptides. Antimicrobial peptides are broad-spectrum antimicrobial agents against Gram-negative and Gram-positive bacterial strains, viruses, and fungi. They have not yet been widely used in clinical settings, and on the other hand, AMP coatings still face challenges such as loss of antimicrobial activity, non-specific binding, and changes in peptide orientation. Additionally, researchers are investigating the use of hydrogels as carriers for loading antimicrobial agents derived from furanones on urinary catheters, which show better antimicrobial effects compared to traditional Foley catheters. In reality, most antimicrobial catheters currently under investigation rely on antimicrobial drugs (5). These polymers play an important role in improving the bioavailability, bioactivity, drug delivery control, and reducing the side effects of drugs and nanoparticles by encapsulating them. This drug delivery system is widely used in various applications in nanomedicine and also has the potential to prevent microbial biofilm formation. Various types of nanoparticles, such as micelles, nanospheres, and liposomes, have been coated on the surface of catheters, which can control and stabilize antibacterial or antibiotic compounds. It has been reported that chlorhexidine, gentamicin, vancomycin, and chlorhexidine are encapsulated in nano-carrier systems. Various methods have been used to coat medical implants, including urinary catheters, with nanoparticles. Among these methods, layer-by-layer (LbL) coating and spray layering are used to coat different compounds on medical implants and surfaces (4).

In fact, the field of nanotechnology deals with the creation and use of materials with nanoscale dimensions. Nanoparticles have unique properties due to their high surface-to-volume ratio. Several chemical and physical methods have been used to

prepare zinc oxide nanoparticles (ZnO-NPs). However, bio-based methods using green or natural approaches in various substrates (such as plant extracts, enzymes, and microorganisms) can offer long-term environmental benefits and lower costs compared to chemical and/or physical methods. ZnO-NPs are currently being studied as antibacterial agents in nanoscale and micrometer formulations. Today, the biocompatibility and biomedical properties of ZnO nanoparticles have improved compared to their traditional counterparts, making ZnO nanoparticles excellent antibacterial agents. Additionally, these ZnO nanoparticles are useful in wound healing processes and biosensing components for detecting small portions of biomarkers associated with various disorders. It has also been observed that ZnO-NPs have the potential to deliver drugs. ZnO nanoparticles have a high potential as a powerful biological agent (6).

Studies have shown that the combination of Ag and ZnO nanoparticles has greater biocompatibility and antibacterial properties compared to the use of either particle alone. Therefore, with the aim of benefiting from the positive features of both nanoparticles, the higher biocompatibility and lower cost of zinc oxide compared to silver nanoparticles, as well as the superior antibacterial properties of silver nanoparticles compared to zinc oxide nanoparticles, a combination of Ag/ZnO nanoparticles was used to produce an antibacterial lubricant gel for coating Foley catheters. The antibacterial effect under laboratory conditions, as well as the irritability and desensitization of the lubricant infused with antibacterial nanoparticles to prevent microbial biofilm formation and reduce the adhesion of pathogenic bacteria to the external surface of the catheter during catheterization, were investigated.

Materials and Methods

Synthesis of Silver nanostructure and Zinc Oxide

To prepare colloidal nanoparticles using the chemical reduction method, a 0.5 millimolar solution of silver nitrate was first prepared with a 0.02 molar solution of sodium citrate. Then, the sodium citrate solution was added to the silver nitrate. In the next step, a 0.01 molar solution of sodium borohydride (Merk, Germany) was prepared and added to the container containing sodium citrate and silver nitrate. The final solution was placed on a magnetic stirrer for 10 minutes. Additionally, to prepare the colloidal zinc oxide nanoparticles, 0.2 molar zinc acetate dihydrate was mixed with methanol, and then this solution was placed on a magnetic stirrer. In another container, 1.2 molar sodium hydroxide was dissolved in methanol. The zinc acetate solution was added to the sodium hydroxide solution. The final solution was stirred on the magnetic stirrer for three hours. A centrifuge was used to separate macroscopic-sized nanoparticles from microscopic-sized ones.

Preparation of a lubricating gel containing a silver/zinc oxide colloidal nanostructure and lidocaine

To prepare approximately ten kilograms of a lubricating gel containing lidocaine (2%) and antibacterial nanostructure, lidocaine powder was mixed thoroughly with a quantity of lubricating gel (from Pahliz Teb Shafagh) in a mixer until the desired weight was achieved and a uniform, homogeneous mixture was obtained. Then, a suspension of antibacterial nanostructures was agitated in a container to mix the contents and eliminate the biphasic environment, allowing the settled particles to remain suspended. Approximately half a kilogram of the antibacterial nanostructure suspension was weighed in a beaker and immediately added to the mixer containing the lubricating gel and 2% lidocaine. The mixing

process in the tank continued for about one hour until a uniform mixture was formed with the lubricating gel and 2% lidocaine. After a uniform mixture of the lubricating gel and 2% lidocaine containing antibacterial nanostructures was formed, packaging was carried out.

Identification and Estimation of Nanostructure Dimensions

The PDI index was used to determine the particle size distribution and obtain the zeta potential of the nanostructure using DLS (Stereoscope-IN A-ONE Enc., Korea) spectrometry. The approximate dimensions and structure of the nanostructures were assessed using TEM images. Additionally, the precise concentration of nanostructures in each solution was examined and calculated using the ICP test (ICP-OES 730-ES, Varian).

Investigation of Antibacterial Properties

The evaluation of the zone of inhibition diameter by the disk diffusion method was conducted by saturating blank discs with a lubricant gel containing colloidal nanostructures and lidocaine. To perform the disk diffusion, a microbial suspension equivalent to half McFarland from 18-hour colonies was prepared. Then, using a sterile swab, the microbial suspension was inoculated on the surface of Muller-Hinton agar, and after 15 minutes of inoculation, the blank discs were placed on the surface of the medium. After another 15 minutes of placing the discs, the plates were incubated at a temperature of 35-37 degrees Celsius for 16-18 hours. The diameter of the zone of inhibition was measured using a ruler.

To investigate the antibacterial effects of silver/zinc oxide colloidal nanostructures against standard strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the Tube-Dilution method was used. In this method, different dilutions of the silver/zinc oxide colloidal nanostructures were prepared with a standard McFarland broth equivalent to 10^8 × 1.5 bacteria. Approximately 1 milliliter of the growth medium was mixed with 1 milliliter of the nanostructure to create the dilution. Then, 0.1 milliliters of the bacterial suspension were added to the various dilutions of the nanostructure in Mueller Hinton broth, and the sensitivity of the bacteria was assessed with the naked eye after incubation. The lowest concentration of nanostructure that resulted in a significant visible growth was indicative of the bacteriostatic effect of the substance and was referred to as the Minimum Inhibitory Concentration (MIC). After obtaining the MIC, the bactericidal concentration of the nanostructure, known as Minimum Bactericidal Concentration (MBC), was also determined. This was done by taking 0.1 milliliters from each of the prepared tubes and transferring it to solid growth medium. After incubation, if the environment showed 99.9% fewer colonies than the control plate, the MBC was considered achieved.

Animal Study

The level of irritability to the medical lubricating gel (containing antibacterial nanostructure) was tested in 6 albino rabbits using 0.1 milliliters of a 1:1 dilution in water (emulsion) in the conjunctival sac of the left eye of the rabbits. In this test, 0.1 milliliters of water was used as a control (control group) in the conjunctival sac of the right eye of the rabbits. Both eyes of three out of the six treated animals were washed one minute after application using 20 milliliters of water in each case. For the washing process, warm tap water (37 ºC) with a pH of 7.3 and a hardness of 21 OdH was used. The results were evaluated using a magnifying glass after 10, 60, 180, and 330 minutes post-treatment and after 24, 48, and 72 hours. After completing these evaluations, the treated eyes were rinsed with physiological saline solution.

Sensitization Test

The sensitization test was conducted in vivo according to the international standard ISO 10993-10 using the Guinea Pig Maximization Test (GPMT) method. The level of sensitization of the product was determined by injecting the sample into the skin of guinea pigs, and then evaluated specialist according to the amount of edema and erythema on the animal's skin. To prepare the extract, for each 0.2 milliliters of the test sample, 1 milliliter of normal saline was added to the container containing the sample and was kept in an incubator at a temperature of 37 ºC for a period of ±72 hours. A 30% concentration was prepared from the sample extract. To prepare a negative control sample, 1 milliliter of normal saline was kept in the incubator at a temperature of 37 ºC for a period of ±72 hours. Sample preparation for injection was carried out as follows:
Site A: A 50:50 volume ratio stable emulsion of Freund's complete adjuvant mixed with sodium chloride injection (0.9%).
Site B: the test sample (undiluted extract): inject the control animals with the solvent alone.

Site C: the test sample at the concentration used at site B emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%); inject the control animals with the emulsion of the blank liquid with adjuvant.

For the test, 10 male guinea pigs weighing approximately 300 grams were observed as test animals for 1 week prior to the test. 5 male guinea pigs weighing approximately 300 grams were observed as control animals for 1 week prior to the test. The backs of all guinea pigs were shaved ±24 hours before the test. Before conducting the test, all animals were checked for health status.

The test is conducted in two phases. The injection areas are shown in Figure 1 In Phase One (Induction phase) on Day One: 0.1 milliliters of the prepared solutions were injected intradermally into the lower back of all test and control guinea pigs at injection sites A, B, and C. On Day Six: 1 milliliter of sodium dodecyl sulfate (10%) solution was added to the lower back of all animals. On Day Seven: 0.5 milliliters of the solution from site B was added to an 8 square centimeter patch and bandaged in the injection area of all animals, and after 2±24 hours, the dressings of the animals were removed. In Phase Two (Challenge phase) on Day Twenty-One: 0.5 milliliters of the sample extract was added to an 8 square centimeter patch and bandaged on the right side of all animals. 0.5 milliliters of normal saline was added to an 8 square centimeter patch and bandaged on the left side of the animals. After 2±24 hours, the bandages were removed. After 2±24 and 2±48 hours of removing the bandages, the backs of the animals were assessed for edema and erythema using established criteria. The sensitization test uses a four-point grading scale to evaluate skin reactions. A grade 0 indicates No reaction. Grade 1 is Weakly positive reaction (usually characterized by mild erythema across most of the treatment site). Grade 2 represents Moderate positive reaction (usually distinct erythema possibly spreading beyond the treatment site). The most severe reaction, grade 3, is characterized by Strongly positive reaction (strong, often spreading erythema with odema) (7). This standardized scale allows for consistent assessment of potential skin sensitization responses. In this test, if the test sample has a sensitivity of zero compared to the negative control sample, it is considered non-sensitizing. If the test sample has a sensitivity of one or more compared to the negative control sample, it is considered sensitizing.

Results

Dynamic light scattering (DLS) measurements were performed using a Stereoscope-IN A-ONE (Enc., Korea) instrument. The intensity distribution spectra revealed that the dynamic diameter of the colloidal nanostructures of silver and zinc oxide were approximately 24.4 nm and 52.48 nm, respectively. Additionally, the

polydispersity index was calculated through the size distribution of the silver and zinc oxide nanostructures to be approximately 1 and 0.667, respectively. The closer the values of the PDI are to 1, the more it indicates the monodispersity of the synthesized nanostructure, whereas the further the obtained values for PDI are from 1, the more it indicates the polydispersity of the synthesized nanostructure. The results showed that the population of silver and zinc oxide nanostructures separately demonstrated a homogeneous state. Furthermore, the results of the zeta test calculated the zeta potential of silver and zinc oxide nanostructures to be -20.7 mv and -0.709 mv, respectively. A more negative zeta potential helps the silver and zinc oxide nanostructures repel each other more, leading to long-term stability and preventing the agglomeration of the silver and zinc oxide nanostructures (Figure 2).

The concentration of the synthesized nanostructure in each of the colloidal solutions was examined and calculated using the ICP test. Based on the results, the concentration of nanostructure for the colloidal solutions of silver nanostructure and zinc oxide nanostructure were determined to be 25 and 970 ppm, respectively. (Table 1).

The structures of silver and zinc oxide nanostructures were analyzed using electron microscope images (TEM). The TEM images revealed that the silver nanostructure are spherical in shape with a smooth surface morphology, while the zinc oxide nanostructure are agglomerated and have a clustered appearance. According to the TEM images, the average size of the silver nanostructures was estimated to be approximately 3.14 ± 13 nanometers (Figure 3-A), and the zinc oxide nanostructures were estimated to be around 0.88 ± 14.16 nanometers (Figure 3-B).

The results of antimicrobial testing for silver nanostructure (AgNPs) revealed very weak antibacterial properties against the standard strain of *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) of AgNPs was calculated to be >12.5 µg/mL, while the minimum bactericidal concentration (MBC) was also >12.5 µg/mL, indicating low efficacy of AgNPs at various dilutions against P. aeruginosa.

Similarly, AgNPs exhibited very weak antibacterial activity against the standard strain of *Staphylococcus aureus*, with an MIC >12.5 µg/mL and an MBC of approximately >12.5 µg/mL, further confirming their limited antibacterial effect at different concentrations (Table 2).

In contrast, zinc oxide nanostructure (ZnO NPs) demonstrated relatively strong antibacterial activity against P. aeruginosa, with an MIC of ~0.203 µg/mL (Cavity: 8th, 1/256 dilution) and an MBC of ~1.62 µg/mL (Cavity: 5th, 1/32 dilution), suggesting acceptable efficacy at various concentrations.

Additionally, ZnO NPs showed strong antibacterial effects against *S. aureus*, with an MIC of ~0.406 µg/mL (Cavity: 7th, 1/128 dilution) and an MBC of ~1.62 µg/mL (Cavity: 5th, 1/32 dilution), indicating their potent antimicrobial activity (Table 2).

The Ag-ZnO hybrid nanostructure displayed notable antibacterial effects against P. aeruginosa, with an MIC of ~0.62/100 µg/mL (Cavity: 3rd, 1/16 dilution) and an MBC of ~2.5/400 µg/mL (Cavity: 1st, 1/2 dilution), demonstrating acceptable inhibitory and bactericidal activity.

Remarkably, the hybrid nanostructure exhibited extremely strong antibacterial activity against *S. aureus*, with an MIC of ~0.02/3.12 µg/mL (Cavity: 8th, 1/256 dilution) and an MBC of ~0.31/50 µg/mL (Cavity: 4th, 1/16 dilution), highlighting their potent antimicrobial efficacy at various dilutions (Table 2, Figure 4).

The ocular irritation test is a standardized, quantitative acute eye irritation assay used to evaluate the irritant potential of detergents and other medical consumer products, where the test substance is applied to a semi-permeable membrane ("ocular membrane") mimicking the outer corneal epithelium tissue layer.

Table 1. Elemental analysis using ICP-OES (730-ES, Varian) for silver nanostructures and zinc oxide nanoparticles.

Elemental analysis test using the ICP-OES 730-ES instrument (Varian)			
lab.NO.	Sample Labels	Element	Wavelength
		Zn	334.502
	Blank (ppm)	0	
	ZnO nanostructures (ppm)	970	
	Silver nanostructures (ppm)	25	
Note: Elemental concentrations are reported in micrograms per milliliter (µg/mL).			

Table 2. Evaluation of antimicrobial activity of Ag, ZnO, and Ag-ZnO hybrid nanostructures against reference strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* employing disk diffusion and microdilution assays.

		DIZ (mm)	**MIC (µg/ml)**	**MBC (µg/ml)**
Ag NPs	*Psudomonas aeruginosa*	0	≥12.5	≥12.5
	Staphylococcus aureus	0	≥12.5	≥12.5
ZnO NPs	*Psudomonas aeruginosa*	10	≥500	≥500
	Staphylococcus aureus	10	2 (Cavity: 9th, 1/512)	3.125 (Cavity: 4th 1/64)
AgZnO NPs	*Psudomonas aeruginosa*	18	100/0.62 (Cavity: 3th, 1/16)	400/2.5 (Cavity: 1th, 1/2)
	Staphylococcus aureus	15	3.12/0.02 (Cavity: 8th, 1/256)	50/0.31 (Cavity: 4th, 1/16)

Table 3. The sensitization test according to the international standard ISO 10993-10 using the Guinea Pig Maximization Test (GPMT) method.

Test results	**Test method**	**Test type**
At 30% concentration shows no sensitization	ISO 10993-10:2010 guinea pig maximization test (GPMT)	Sensitization test on sample extract

group	**Animal number**	**Time after removal of the patch**		**Total**
		24 ± 2 hours	**48 ± 2 hours**	
Test group	**1**	**0**	**0**	**0**
	2	**0**	**0**	
	3	**0**	**0**	
	4	**0**	**0**	
	5	**0**	**0**	
	6	**0**	**0**	

	7	**0**	**0**	
	8	**0**	**0**	
	9	**0**	**0**	
	10	**0**	**0**	
control	**11**	**0**	**0**	**0**
	12	**0**	**0**	
	13	**0**	**0**	
	14	**0**	**0**	
	15	**0**	**0**	

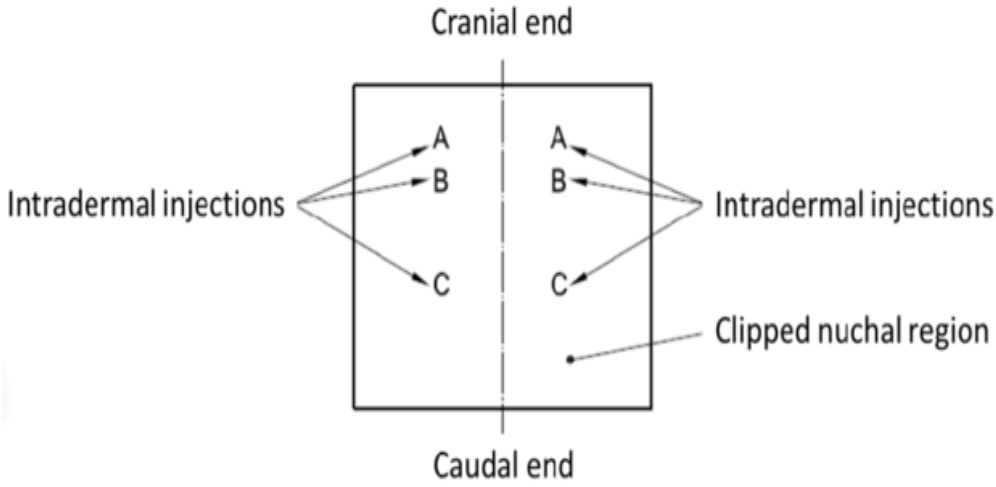

Fig 1. Intradermal injection sites according to the international standard ISO 10993-10 using the Guinea Pig Maximization Test (GPMT) method.

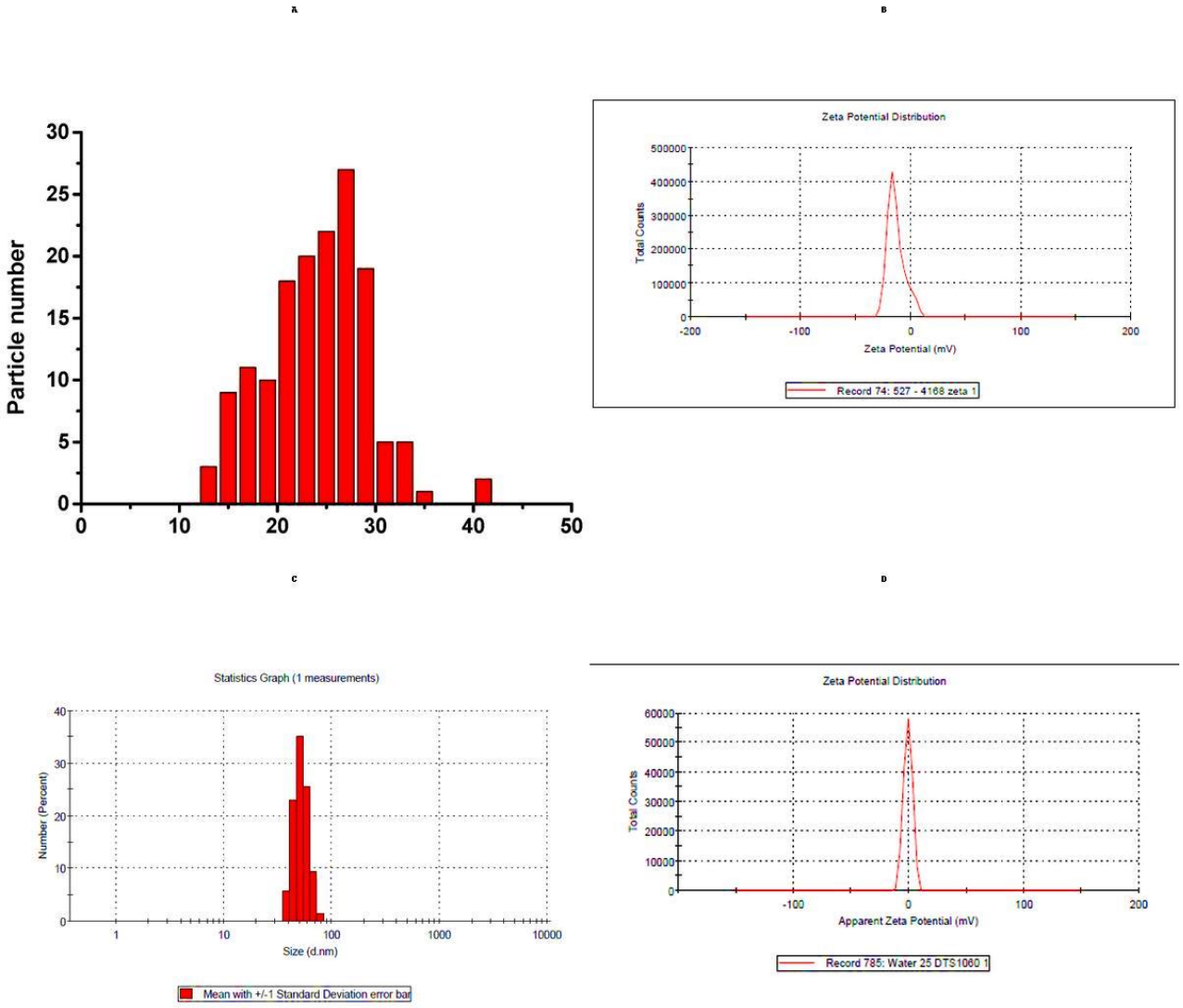

Fig 2. Dynamic diameter range of silver colloidal nanostructures (A), Zeta potential of silver nanostructures (B), Dynamic diameter range of zinc oxide colloidal nanostructures (C), Zeta potential of zinc oxide nanostructures (D).

As the sample diffuses through the ocular membrane, it contacts a macromolecular matrix containing a structural component resembling corneal macromolecules in organization and a biomarker component that predicts molecular damage related to immune response and cell viability. Under these test conditions, the peak irritation indices were 3.5 for rinsed eyes after 180 or 330 minutes, 6.5 for non-rinsed eyes after 180 or 330 minutes, and 4.5 as the mean value across

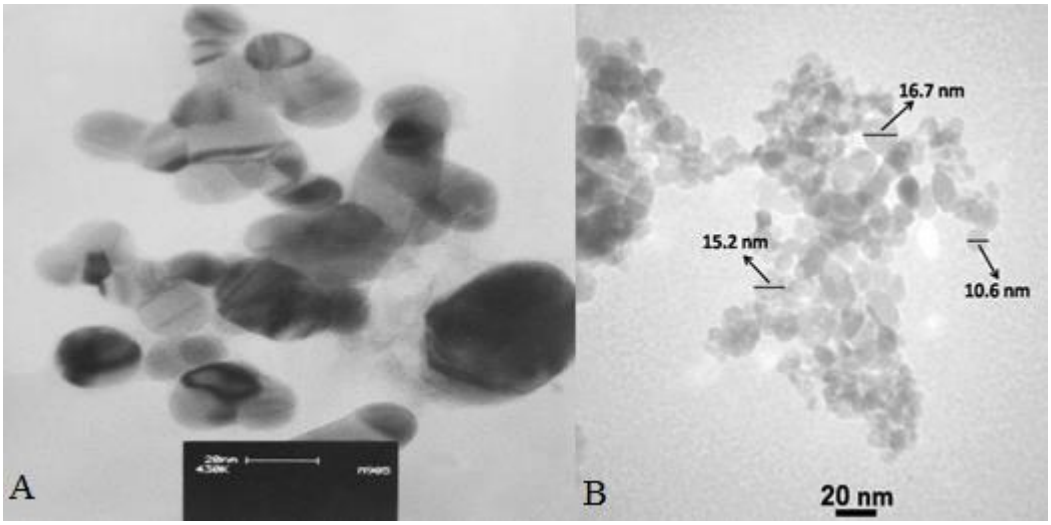

Fig 3. TEM image of silver nanostructures at 460K magnification with a 20 nm scale bar (A), TEM image of zinc oxide nanostructures at 200K magnification with a 20 nm scale bar (B).

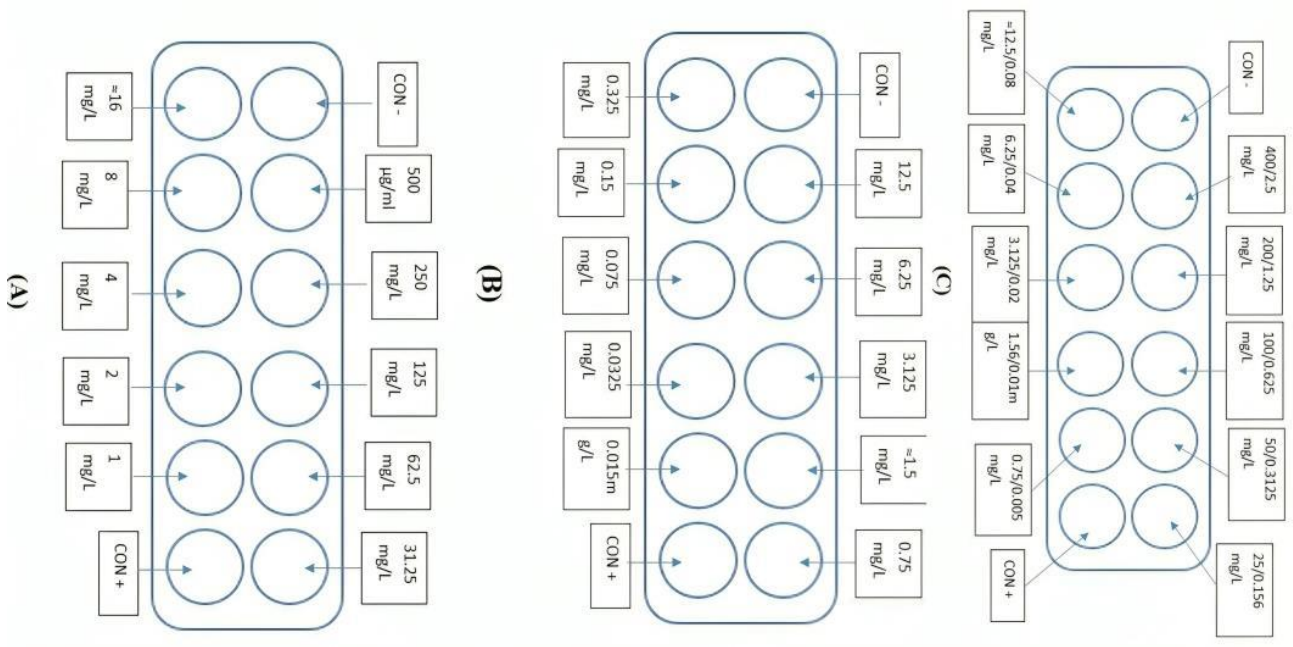

Fig 4. Zinc oxide nanoparticles (A), Silver oxide nanoparticles (B), Silver-Zinc oxide nanoparticles (C).

all eyes after 180 or 330 minutes. According to Report No. P.0.14155/6451 from the Cellular and Animal Laboratory of Tehran University of Medical Sciences' Faculty of Pharmacy, supervised by Prof. Dr. Seyed Nasser Ostad (Professor of Toxicology and Pharmacology at TUMS, Medical Council ID: 2341), the antibacterial nanostructure -containing medical

lubricating gel can be classified as non-irritating to laboratory animal mucosal membranes.

The sensitization test evaluated the compatibility of the test sample (an antibacterial medical lubricating gel containing nanostructure) at a 30% concentration. The results demonstrated that the sample extract elicited no edema or erythema responses (total score: 0), indicating the absence of sensitization potential at this concentration (Table 3).

Discussion

In this study, silver nanostructure (AgNPs), zinc oxide nanostructure (ZnO NPs), and 2% lidocaine were synthesized, followed by mixing at a volumetric ratio of 2:8 (AgNPs:ZnO NPs). The constituent elements were characterized, and parameters including particle size distribution, zeta potential, and concentration were estimated using dynamic light scattering (DLS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and transmission electron microscopy (TEM). Subsequently, the inhibitory zone diameters of standard strains (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) were evaluated against the nanostructure via the disk diffusion method. Antibacterial effects were further quantified using the microplate dilution (broth microdilution) method. For assessing sensitization potential and irritancy, in vivo animal tests were conducted on the AgNPs, ZnO NPs, and 2% lidocaine formulations.

The intensity distribution spectra revealed dynamic diameter ranges of approximately 24.4 nm for silver nanostructure (AgNPs) and 52.48 nm for zinc oxide nanostructure (ZnO NPs). The polydispersity index (PDI) was calculated as 1 for AgNPs and 0.667 for ZnO NPs, indicating monodisperse distribution characteristics, which suggests homogeneous populations of individually dispersed synthesized nanostructure. Zeta potential measurements yielded values of -20.7 mV for AgNPs and -0.709 mV for ZnO NPs. Quantitative analysis determined colloidal concentrations of 25 ppm for AgNPs and 70 ppm for ZnO NPs in aqueous solvent. TEM imaging demonstrated that AgNPs exhibited spherical morphology with smooth surfaces, while ZnO NPs displayed aggregated, clustered configurations. Particle size analysis using Digimizer software estimated mean diameters of 13 ± 3.14 nm for AgNPs and 14.16 ± 0.88 nm for ZnO NPs.

Lethongkam S et al, synthesized silver nanostructure-coated Foley urinary catheters using *Eucalyptus camaldulensis* leaf extract, reporting particle sizes ranging from 20 to 120 nm via scanning electron microscopy (SEM) (8). In a parallel study, Roe D et al, developed antimicrobial silver nanostructure coatings for Foley catheters, producing distinct spherical particles with diameters of 3-18 nm (mean: 10.7 nm) (9). The colloidal silver nanostructures fabricated in our current study exhibited dimensions comparable to those reported by both Roe et al. (9) and Lethongkam S et al. (8). Goda R M's team alternatively synthesized silver nanostructure using *Pistacia lentiscus* (mastic) extract, demonstrating spherical morphology with smooth surfaces and particle sizes predominantly between 15-25 nm (10). The observed minor size variations among studies may be attributed to: (i) divergent synthesis methodologies, (ii) laboratory condition variability, (iii) reagent purity differences, and (iv) technical expertise in optimizing protocol-specific parameters.

Kaijun Li et al. demonstrated that hydrophilic lubricious coatings with antifouling properties can prevent biofilm formation on urological devices such as ureteral stents (US), thereby reducing risks of kidney stone formation and infectious contamination. However, the long-term antibacterial efficacy of these smart lubricants remains under investigation.

The research team successfully developed a hydrophilic lubricious coating (SA-PU/PVP) capable of urease-responsive antibiotic release, achieved through sulfanilamide-polyurethane

(SA-PU) antibiotic delivery within a hydrophilic polyvinylpyrrolidone (PVP) matrix. During the study period, the hydrophilic PVP chains rapidly absorbed urine at the coated surface, forming an antibacterial lubricious layer that reduced bacterial protein adhesion by >90%.

Key findings revealed that increased bacterial colonization triggers urease production, which subsequently cleaves urea bonds in the SA-PU/PVP coating, initiating SA antibiotic release. This mechanism ultimately prevents microbial biofilm formation and kidney stone development. Seven-week porcine model trials demonstrated superior performance of these smart lubricious coatings on hydrophilic stents compared to commercial alternatives (11). Notably, while Kaijun Li et al. employed sulfanilamide antibiotic in their design, the growing global challenge of antimicrobial resistance suggests that supplementary antibacterial nanostructure could enhance efficacy (11). In our current study, disk diffusion, MIC, and MBC assays revealed weak antibacterial properties of silver nanostructure (AgNPs) against standard strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

This contrasts sharply with findings by Lethongkam S et al., who reported that AgNP-coated catheters significantly inhibited microbial adhesion and biofilm formation in human urine-mimicking environments during infection exposure periods (8). Their work posited that AgNP-coated catheters exhibit broad-spectrum antimicrobial activity against catheter-associated urinary tract infection pathogens (8). The observed discrepancy may stem from: Differences in nanostructure synthesis methods, variations in urinary simulation media composition, distinct bacterial exposure protocols, potential nanostructure aggregation in physiological fluids.

In contrast to our results, Roe D et al. demonstrated that Foley catheters coated with non-radioactive silver nanostructure (AgNPs) exhibited significant antimicrobial activity, inhibiting cellular growth and biofilm formation for ≥72 hours, with 67% suppression of *Pseudomonas aeruginosa* and near-complete inhibition (~100%) of *Escherichia coli* and *Staphylococcus aureus* colonization (9).

In contrast, zinc oxide nanostructures demonstrated relatively strong antibacterial properties against standard strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. By mixing silver nanostructures with zinc oxide nanostructures, the antibacterial effects were enhanced, showing a synergistic antimicrobial activity. According to the results, the mixture of silver and zinc oxide nanostructures inhibited bacterial growth of *P. aeruginosa* at a concentration of 0.62/100 µg/mL and exhibited bactericidal properties at 2.5/400 µg/mL. For *S. aureus*, the mixture inhibited growth at 0.02/3.12 µg/mL and showed bactericidal effects at 0.31/50 µg/mL. Wu K et al, investigated the antimicrobial activity and cellular compatibility of silver nanostructure-coated catheters, confirming their ability to prevent colonization of live bacteria using the Zol test and fluorescence staining. The Zol test is based on the leaching of silver ions from the surface. They found that bacterial growth inhibition depends on sufficient concentration of silver ions in the aqueous environment (12). Similarly, in our study, the concentration of silver and zinc oxide ions was measured by ICP, suggesting that metal ion concentration significantly affects the nanostructure antibacterial effects. However, unlike our findings, antibacterial tests on *S. aureus* showed clear inhibition zones (0.2-0.35 mm) around silver nanostructure-coated catheters, indicating effective antibacterial activity. Wu k et al. demonstrated that antibacterial efficacy could be modulated by adjusting nanostructure coating density (12). Additionally, contrary to our results, Goda R M et al. found that silver nanostructure-coated catheters significantly prevented colonization by antibiotic-resistant Gram-positive (*S. epidermidis* and *S. aureus*) and Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and

P. aeruginosa), with silver nanostructure being more active against Gram-negative bacteria (10). Consistent with our findings, Goda R M et al. observed that the inhibitory effect lasted for 72 hours (10).

In the present study, disk diffusion test results against *Staphylococcus aureus* revealed a distinct 15 mm inhibition zone around blank disks coated with silver and zinc oxide nanostructures, demonstrating their effective antibacterial activity. Similarly, testing against *Pseudomonas aeruginosa* showed an 18 mm inhibition zone around coated disks, indicating strong antibacterial properties. However, our study found that silver nanoparticles exhibited weaker antibacterial effects compared to zinc oxide nanoparticles. Notably in Wu K. et al study, mixing silver and zinc oxide nanoparticles at a 2:8 volumetric ratio enhanced antibacterial activity, showing synergistic effects against both Gram-positive and Gram-negative bacteria (12). We attribute this to zinc oxide nanoparticles releasing more than double the concentration of divalent ions (900 ppm) in aqueous environments compared to silver nanoparticles (25 ppm). As previously explained, the nanoparticle concentration per milliliter of suspension plays a decisive role in the antibacterial mechanism.

The irritability test in laboratory animals showed that the medical lubricating gel (containing antibacterial nanoparticles) caused no mucosal irritation. Additionally, sensitization testing demonstrated zero edema or erythema response to the 30% concentration antibacterial nanoparticle gel, confirming its non-sensitizing properties at this concentration.

Conclusion

The active formulation consisting of mixed silver and zinc oxide nanostructures combined with a medical lubricant containing 4% lidocaine demonstrates effective antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while exhibiting no irritant or sensitizing properties in laboratory animals.

Acknowledgements

We would like to thank Guilan University of Medical Sciences for its assistance and guidance in this research.

Funding Information

The author(s) received no specific funding for this work.

Ethics approval and consent to participate

A lubricant containing antibacterial nanoparticles and lidocaine was applied to the conjunctival sac of the left eye in an albino rabbit and on the skin of a male guinea pig, following approval by the Ethics Committee of Guilan University of Medical Sciences (Ethical Code: IR.GUMS.REC.1402.341). Currently, the use of medical lubricants has no side effects and has been approved by the Food and Drug Administration and the General Department of Medical Equipment. Additionally, based on previous studies, zinc oxide and silver nanoparticles (at the intended concentration in this project) show no cytotoxic effects. In compliance with medical ethics, efforts have been made to use the minimum sample size when employing laboratory animals for this study.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

References

1. Nandini MS, Madhusudan K. Bacteriological profile of catheter associated urinary tract infection and its antimicrobial susceptibility

pattern in a tertiary care hospital. *J Pharm Sci Res* 2016; **8**(4):204.
2. Magill SS, Edwards JR, Bamberg W, et al. Multistate point-prevalence survey of health care–associated infections. *New Eng J Med* 2014; **370**(13):1198-208.
3. Stickler DJ, Zimakoff J. Complications of urinary tract infections associated with devices used for long-term bladder management. *J Hos Infect* 1994; **28**(3):177-94.
4. Dawson KA, Salvati A, Lynch I. Nanoparticles reconstruct lipids. *Nat Nanotechnol* 2009; **4**(2):84-5.
5. Crabtree JH, Burchette RJ, Siddiqi RA, et al. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. *Perit Dial Int* 2003; **23**(4):368-74.
6. Islam F, Shohag S, Uddin MJ, et al. Exploring the journey of zinc oxide nanoparticles (ZnO-NPs) toward biomedical applications. *Materials* 2022; **15**(6):2160.
7. Lu W, Luo H, Wu Y, et al. Preparation and characterization of a metered dose transdermal spray for testosterone. *Acta Pharm Sin B* 2013; **3**(6):392-9.
8. Lethongkam S, Paosen S, Bilhman S, et al. Eucalyptus-mediated synthesized silver nanoparticles-coated urinary catheter inhibits microbial migration and biofilm formation. *Nanomaterials* 2022; **12**(22):4059.
9. Roe D, Karandikar B, Bonn-Savage N, et al. Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *J Antimicrob Chemother* 2008; **61**(4):869–76.
10. Goda RM, El-Baz AM, Khalaf EM, et al. Combating bacterial biofilm formation in urinary catheter by green silver nanoparticle. *Antibiotics* 2022; **11**(4):495.
11. Li K, Tang H, Peng J, et al. Smart lubricant coating with urease-responsive antibacterial functions for ureteral stents to inhibit infectious encrustation. *Adv Funct Mater* 2024; **34**(2):2307760.
12. Wu K, Yang Y, Zhang Y, et al. Antimicrobial activity and cytocompatibility of silver nanoparticles coated catheters via a biomimetic surface functionalization strategy. *Int J Nanomedicine* 2015;7241-52.